c) culturing the transformed explant in a media comprising an apical dominance inhibitor selected from the group consisting of dikegulac, methyl laurate and octadecyl-polyethoxyethanol to induce bud or shoot formation from the transformed explant; and

d) rooting the transformed explant containing buds or shoots to produce a transgenic plant.

20. (amended) A method for the regeneration of a transgenic plant comprising the steps of:

a) providing an explant of a plant comprising a shoot meristem or primordia;

b) culturing the explant in a media comprising an apical dominance inhibitor selected from the group consisting of dikegulac, methyl laurate and octadecyl-polyethoxyethanol to induce bud or shoot formation from the explant;

c) introducing a recombinant DNA vector into the explant to generate a transformed explant; and

d) rooting the transformed explant containing buds or shoots to produce a transgenic plant.

REMARKS

Attached hereto is a marked-up version of the changes made to the claims by the current amendments. The attached page is captioned "**Version With Markings To Show Changes Made.**"

Restriction Requirement

Applicant affirms its election of Invention I, with traverse, as made on 10 September 2002. Claims 21-23 have been cancelled without prejudice as being drawn to a non-elected invention and Applicants reserve the right to pursue the invention of these claims in further divisional applications.

Objection to the Specification

A copy of the electronic filing receipt is enclosed herewith that shows Table 1 having been transmitted with the application as filed. When applicants printed a copy of the application from their

file copy, Table 1 readily printed. A copy of Table 1 is provided with this Response for the examiner's file, and Applicants request that it be entered into the specification in paragraph 0093. If the Office would like the electronic tif file of Table 1, please advise the undersigned. Also enclosed is a copy of the corrected drawings that have been sent to the attention of the Official Draftsperson on even date herewith.

Rejections based on 35 U.S.C. §112

Claims 1, 8, 9, 15, 16, 17, 19 and 20 stand rejected under 35 USC 112, second paragraph for the reasons stated on pp. 3-4 of the Office Action. In view of the amendments to the claims made above, Applicants believe that the rejections have been overcome and request reconsideration and withdrawal of the 112, second paragraph rejections.

Claim 13 stands rejected under 35 USC 112, first paragraph for the reasons stated on pp. 4-5 of the Office Action. Applicants respectfully traverse this rejection and request reconsideration in view of the amended claim and the following remarks. The specific cotton lines presented in claim 13 are merely representative of commercial or elite cotton lines that could be used in conjunction with the present invention. The invention is in no way limited to these particular lines recited in the claim and reference to them has been deleted from the claim.

Claim 18 stands rejected under 35 USC 112, first paragraph as not being enabling for any modification of the specific culture media described therein. These media and modifications thereto are well known to those skilled in the art and have been available in the art for years. One of skill in the art of tissue culture would easily know what minor modifications could be made to any of these media. Applicants' invention is not dependent upon the base media in the culture. Any media that supports plant tissue growth and development can be used, as the Applicants point out in paragraph 0067 of the application. Notwithstanding this knowledge, however, Applicants have cancelled this language in claim 18 as it would be redundant of the media claimed in claim 1.

Rejections based on 35 U.S.C. § 102(b)

The Office has presented four separate 102(b) rejections. Claims 1-4, 11-12, 16 and 18 were rejected under 35 U.S.C. 102(b) as being anticipated by Bajaj et al. 1986. Claims 1, 17 and 20 were rejected under 35 U.S.C. 102(b) as being anticipated by Smith et al. 1992. Claims 1 and 15 were rejected under 35 U.S.C. 102(b) as being anticipated by Morre et al. 1998. Claims 1 and 14 were rejected under 35 U.S.C. 102(b) as being anticipated by Mohamed-Yasseen et al. 1990. Applicants respectfully traverse

these rejections and request reconsideration in view of the following remarks and claim amendments. Because the Office has presented a similar argument in support of each of these rejections, Applicants believe it is proper to address them as a group. For all of these 102 rejections, the Office has taken the position that the term "apical dominance inhibitor" includes "any compound that either negatively or positively is involved in adventitious meristem production." Applicants' request reconsideration of this interpretation and request that the Office consider the specific definition provided in the specification and the manner in which the term is used in the claims. The Office has adopted an overly broad interpretation of this term that is inconsistent with the term and the invention as described in the application. As stated in paragraphs 0006, 0007, 0016 in the application, the apical dominance inhibitor is a non-cytokinin compound to be used instead of a cytokinin in plant tissue culture media to promote adventitious meristem production and lateral bud break, i.e., multiple shoots from a single explant. All of the art cited by the Office in its 102 rejections describe media that contain a cytokinin for this purpose. For example, Bajaj et al. described media to induce bud or shoot formation that contained a cytokinin, either kinetin or BA (see pp. 581-583). The only media containing solely IAA and MS salts was the rooting media used in a step in the process that is performed subsequent to bud or shoot formation. Thus, the media asserted to anticipate the claimed invention has IAA and MS salts but is not used to induce bud or shoot formation and there is no indication that such a media would promote adventitious meristem production and lateral bud break. Applicants' claimed invention is drawn to culturing an explant in a media comprising an apical dominance inhibitor (where the compound is a non-cytokinin) to induce bud or shoot formation from the explant and nothing in Bajaj et al. describes such a step in a method for regeneration of a plant. Likewise, Smith et al. describes promoting multiple shoots only with a medium comprising 0.1 mg/L N6-benzyladenine (a cytokinin, see column 4, 4th paragraph,). Therefore this reference does not teach using anything but a cytokinin to get a multiple shoot response. Moreover, Smith et al. do not describe even getting a multiple shoot response (and thus not within the definition of an apical dominance inhibitor) from the cytokinin. Similarly, the Office's assertion that Morre et al.'s use of glucose, MS salts and B5 vitamins are compounds that promote adventitious meristem production is misplaced. In particular it should be noted that Morre et al. supplements its media with "either 0, 1.5, 3 or 6 mg/L of BA" (a cytokinin). Further, when describing its experimental results regarding multiple shoot induction and development, it is stated: "During the second week, axes maintained on medium with BA showed a wider apical area, where numerous leaf and bud primordial started to develop. ... On the other hand, axes cultured on medium without BA and those maintained for two days with the cytokinin

developed just one shoot after 20-25 days of culture." Thus, without a cytokinin, multiple shooting did not occur and therefore the conclusion that an apical dominance inhibitor as defined by Applicants was not present in the media. Finally, In Mohamed-Yasseen et al, the use of N-phenyl, N-1,2,3-thiadiazol-5-yl-urea (also known as thidiazuron (TDZ)), is described which is a cytokinin (see paragraph 0068). Using a cytokinin in the media alone to induce shoot formation clearly falls outside the scope of the claimed invention. In view of all of the foregoing, it is requested that these 102 rejections be withdrawn. For purposes of clarification and for expedited prosecution, Applicant has presented amended claims herein to specific apical dominance inhibitors without prejudice to its right to present claims of the original scope in future continuation applications.

Rejection based on 35 U.S.C. § 103(a)

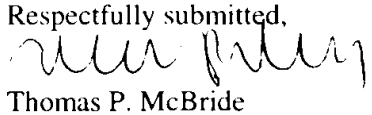
Claims 1-12, 16 and 18 stand rejected under 35 USC §103 (a) as being unpatentable over Bajaj et al., in view of deSilva et al. Applicants respectfully traverse this rejection and request reconsideration in view of the following remarks.

Bajaj et al. discloses a method of regenerating a cotton plant after producing multiple shoots with a cytokinin (as discussed in the previous section). There is no disclosure, teaching or suggestion of any of the recited apical dominance inhibitors in the claims as now amended, nor do they suggest that other compounds other than cytokinins can produce multiple shoots.

deSilva et al. discloses a method of chemical pinching of azalea plants using dikegulac applied to whole plants. No suggestion, teaching or disclosure of the use of an apical dominance inhibitor such as dikegulac in plant tissue culture is provided. The rejection is therefore improper because the cited publications provide no suggestion or motivation to combine the disparate teachings of the references, and there was no reasonable expectation of success based upon the distinct teachings of the cited references. At best the claimed invention may seem "obvious to try" but this is not the proper standard to apply in determining obviousness, nor is a finding of obviousness based on improper hindsight permissible.

The applicants respectfully request reconsideration on the merits of the application as a whole. The Examiner is encouraged to call the undersigned should any further action be required for allowance.

Respectfully submitted,



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June 3, 2003

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

The Claims have been amended as follows:

1. (amended) A method for the regeneration of a plant comprising the steps of:

a) providing a plant explant comprising a shoot meristem or primordia;

b) culturing the explant in a media comprising an apical dominance inhibitor [in a manner inducing] selected from the group consisting of dikegulac, methyl laurate and octadecyl-polyethoxyethanol to induce bud or shoot formation from the explant; and

c) rooting the explants containing buds or shoots to produce a plant.

8. Cancelled

9. (amended) The method of claim 5, wherein the dikegulac is present at a concentration from about 5 to about 5000 mg/L.

13. (amended) The method of claim 12, wherein said cotton plant is [SG747, SG125, HS26, PM2379, DP388, STVL474, DP50, or other] a commercial variety or elite line.

15. (amended) The method of claim 1, wherein said explant is the zygotic embryo or an explant [portion] thereof.

16. (amended) The method of claim 1, wherein said shoot meristem or primordia explant is a node, the cotyledonary node, shoot tip, or an explant [portion] thereof.

17. (amended) The method of claim 1, wherein said shoot meristem or primordia explant is an in vitro-produced shoot, tissue culture, shoot culture, or an explant [portion] thereof.

19. (amended) A method for the regeneration of a transgenic plant comprising the steps of:

a) providing an explant of a plant comprising a shoot meristem or primordia;

b) introducing a recombinant DNA vector into the explant to generate a transformed explant;

c) culturing the transformed explant in a media comprising an apical dominance inhibitor [in a manner inducing] selected from the group consisting of dikegulac, methyl laurate and octadecyl-polyethoxyethanol to induce bud or shoot formation from the transformed explant; and

d) rooting the transformed explant[s] containing buds or shoots to produce a transgenic plant.

20. (amended) [The method of claim 19, wherein the recombinant DNA vector is transformed into the explant after in vitro bud or shoot formation in culture.] A method for the regeneration of a transgenic plant comprising the steps of:

a) providing an explant of a plant comprising a shoot meristem or primordia;

b) culturing the explant in a media comprising an apical dominance inhibitor selected from the group consisting of dikegulac, methyl laurate and octadecyl-polyethoxyethanol to induce bud or shoot formation from the explant;

c) introducing a recombinant DNA vector into the explant to generate a transformed explant; and

d) rooting the transformed explant containing buds or shoots to produce a transgenic plant.

Claims 21-23 are cancelled.

Acknowl[redacted]gment Receipt:

SUBMISSION TYPE: **Utility Patent Filing**
APPLICATION NUMBER: **09683548**
FIRST NAMED INVENTOR: **John Burns**
TITLE OF INVENTION: **Novel Multiple Shoot Proliferation and** Regeneration System for Plants
ATTORNEY DOCKET NUMBER: **38-21(51450)**

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[0088] Magnet-assisted wounding involves using magnetic metal particles and a magnet to produce microwounds in the tissue. The embryos were placed on a stiff medium of Xanthan Gum to firmly hold the explants in a vertical position. Carbonyl iron particles (60-70 mg) in the 1-10 µm size were used (Gredman Ltd., Taiwan). The particles were added to 2 mL of the **Agrobacterium** solution and mixed gently. The suspension can be added to the tissue either in the presence or absence of the magnet. A permanent magnet of neodymium-iron-boron (2x2x1 inch block) was used. Once the suspension is in the petri dish, the petri dish is moved over the surface of the magnet in a circular orbit at approximately 3 revolutions per second for 2 minutes. The orbit should let the target tissue revolve around the center axis of the field and not move over the edge of the magnet pole. At the end of the wounding, remove from the magnetic field and remove tissue to a suitable co-culture medium.

[0089] Vacuum infiltration was performed by covering the explants with - **Agrobacterium** suspension. The mixture was then placed in a vacuum chamber and the house vacuum (~28 mmHg) was applied either steadily or pulsed up to 4 times for 1 hour. The explants were then co-cultured as described below.

[0090] For scalpel wounding, a #11 scalpel blade was used to make several small incisions in the apical region of the embryos. The embryos were then transfered to the **Agrobacterium** suspension and inoculated for 30 minutes and then co-cultured as described below.

[0091] For needle wounding, shader tattoo needles were used to wound the apical meristem and surrounding region. The embryos were inoculated for 30 minutes and then co-cultured.

[0092] After wounding and inoculation, the embryos were co-cultured with the - **Agrobacterium** containing a GUS gene with a promoter (pMON15722; Figure 1) under the following conditions. The co-culture period was on the Xanthan Gum medium with mannitol + sorbitol in petri dishes (of various sizes), for 3 to 5 days at cool conditions (~22-28°C). Following co-culture, the explants were transferred to GD1 medium with 400mg/L Dikegulac-free acid + 1 mg/L IAA + 1 mg/L BAP + 50mg/L kanamycin. Explants were only allowed to grow in this for 1 day and then were sacrificed for GUS expression. Results are shown in Table 1.

[0093]

Table 1. Wounding Parameters

Experiment	Treatment	# Explants	GUS expression (%) in hypocotyl	GUS expression (%) in regeneration zone
1	no wounding	41	4 (9.8)	2 (4.9)
	magnet	88	18 (20.5)	6 (6.8)
	vacuum infilt.	95	16 (5.9)	1 (1.0)
	scalpel	48	10 (20.8)	5 (10.4)
	needles	36	3 (8.3)	0 (0.0)
2	magnet	32	7 (21.9)	3 (9.4)
	vacuum infilt.	30	4 (13.3)	1 (3.3)
	desiccation	40	14 (35.0)	13 (32.5)
3	desiccation	82	39 (47.6)	23 (28.0)
	desiccation post inoculat.	74	37 (50.0)	11 (14.9)
	desiccation pre and post inoculation	132	26 (19.7)	29 (22.0)

[0094] **Example 4**

[0095] **Tissue culture**: Embryo explants are cultured in media in a suitable culture vessel containing 500 mg/L sodium dikegulac (2,3:4,6-di-o-isopropylidene-2-keto-l-gulonic acid), 1 mg/L IAA (indole-3-acetic acid), and 1 mg/L BA (N6-benzyladenine) (herein after referred to as DIB media). Any common plant tissue culture media can be used, including, but not limited to, modified LP medium, DKW medium, GD1 medium, MS/B5 medium, or WPM medium. Embryo axes should be placed apical end up and basal end into the medium so that the radicle is beneath the medium surface. The explants are cultured axenically at approximately 25-28°C under fluorescent lighting at about 10 - 50µE PAR for a 16-hour photoperiod. Typically it takes 5 - 10 weeks for multiple shoots in cotton to develop to a useful size.

[0096] **Elongation and rooting**After multiple shoots are produced, individual shoots, epicotyls, or entire shoot clump developed on the explant are excised and subcultured on the basal medium used above (without the growth regulators, or with exogenous auxin) under the same temperature and light conditions.

[0097] **Example 5**